UNIVERSITY OF GONDAR
COLLEGE OF MEDICINE AND HEALTH SCIENCES
SCHOOL OF BIOMEDICAL AND LABORATORY SCIENCES
DEPARTMENT OF CLINICAL CHEMISTRY

Chronic Kidney Disease and Associated Factors among Highly Active Antiretroviral Therapy Naïve and Experienced HIV Infected Individuals at the University of Gondar Hospital, Gondar, Northwest Ethiopia.

By: Shibihon Debebe

Advisors: KetselaYirdaw (MSc.)

Daniel Asmelash (MSc.)

A thesis submitted to the Department of Clinical Chemistry, School of Biomedical and Laboratory Sciences, College of Medicine and Health Sciences, University of Gondar for partial fulfillment of the requirements for the degree of Master of Science in Clinical Chemistry.

June, 2017
Gondar, Ethiopia

ACKNOWLEDGMENT

My gratitude goes to my advisors, Mr. Ketsela Yirdaw and Mr. Daniel Asmmelash for their massive suggestions, comments, supports and encouragement doing the write up.

I forward special thanks to University of Gondar, School of Biomedical and laboratory Science, Department of Clinical Chemistry for providing me this opportunity.

I am indebted to all staff members at University of Gondar Hospital, Clinical Chemistry Laboratory and for those nurses working in ART clinic for their excellent services that enabled me to conduct this study without any difficulty. To all of my precious friends, I am grateful for you helping me in making this paper a reality.

My deep gratitude also goes to my study subjects who were volunteered to participate in this study.

Table of Contents **Page**

ACKNOWLEDGMENT i

LIST OF TABLES iv

ABBREVIATIONS AND ACRONYMS v

ABSTRACT vii

1. INTRODUCTION 1

1.1 Background 1

1.2. Statement of the problem 3

2. LITERATURE REVIEW 4

3. SIGNIFICANCE OF THE STUDY 7

4. OBJECTIVES OF THE STUDY 8

4.1. General objective 8

4.2. Specific objectives 8

5. METHODS AND MATERIALS 9

5.1. Study area 9

5.2. Study design and period 9

5.3. Population 9

5.3.1. Source population 9

5.3.2. Study population 9

5.4. Inclusion and exclusion criteria 9

5.4.1. Inclusion criteria 9

5.4.2. Exclusion criteria 9

5.5. Study variable 10

5.5.1. Dependent variable 10

5.5.2. Independent variables 10

5.6. Operational definitions 10

5.7. Sample size 11

5.8. Data collection and laboratory methods 11

5.9. Data management and quality control 11
5.10. Data analysis 12
5.11. Ethical consideration 12
5.12. Result dissemination 13

6. RESULTS 14

6.1. Socio demographic characteristics of study participants 14
6.2. Clinical related information among HIV infected study participant 16
6.3. Renal function tests among HIV infected study participant 19
6.4. Comparison of biochemical and other variables between HAART naïve and experienced 20
6.5. Prevalence of CKD among HIV infected study participants 21
6.6. Factors associated with CKD among HIV infected study participants 22

7. DISCUSSION 25

8. LIMITATION AND STRENGTH OF THE STUDY 28

9. CONCLUSION 29

10. RECOMMENDATIONS 29

11. REFERENCES 30

12. ANNEX 35

LIST OF TABLES

Table 1: Socio-demographic characteristics of HIV infected participants. 15
Table 2: HIV related information among HIV infected participants 17
Table 3; Preselected risk factors among HIV infected study participants 18
Table 4:Mean of biochemical results among adult HIV infected participants 19
Table 5: Comparison of biochemical variables between HAART naive and experienced..... 20
Table 6: Prevalence of CKD based on MDRD among HIV infected participants. 21
Table 7: Factors associated with CKD among HAART naive HIV infected participants..... 22
Table 8: Factors associated with CKD among HAART experienced..... 23
Table 9: Factors associated with CKD among HIV infected participants 24

ABBREVIATIONS AND ACRONYMS

AIDS	Acquired Immune Deficiency Syndrome
AKI	Acute Kidney Injury
AOR	Adjusted Odds Ratio
ART	Anti-Retroviral Therapy
ARV	Anti-Retroviral
BMI	Body Mass Index
cART	combined Anti-Retroviral Therapy
CD4	Cluster of Differentiation 4
C-G	Cockcroft-Gault
CKD	Chronic Kidney Disease
COR	Crude Odds Ratio
CrCl	Creatinine Clearance
Cr	Creatinine
DM	Diabetes Mellitus
EDTA	Ethylene Diamine Tetra Acetic acid
eGFR	estimated Glomerular Filtration Rate
ESRD	End Stage Renal Disease
HAART	Highly Active Anti-Retroviral Therapy

HIVAN	Human Immunodeficiency Virus Associated Nephropathy
HIV	Human Immunodeficiency Virus
MDRD	Modification of Diet for Renal Disease
NKF	National Kidney Foundation
NNRTIs	Non Nucleotide Reverse Transcriptase Inhibitors
NRTIs	Nucleotide Reverse Transcriptase Inhibitors
OR	Odds Ratio
SPSS	Statistical Package for Social Science
TDF	Tenofivir Disoproxil Fumarate
WHO	World Health Organization

ABSTRACT

Background: Chronic kidney disease has emerged as one of the primary co-morbid conditions affecting human immunodeficiency virus infected individuals even after highly-active antiretroviral therapy.

Objectives: To assess chronic kidney disease and associated factors among highly active antiretroviral therapy naïve and experienced HIV infected individuals at the University of Gondar Hospital, Gondar, Northwest Ethiopia.

Method: A Hospital based comparative cross-sectional study was carried out at the University of Gondar Hospital from March to May, 2017. Double population proportion formula and convenient sampling technique were used to select 250 study participants. Socio-demographic and clinical data were collected by using pretested semi-structured questionnaire. The collected data were entered into Epi-Info version 3.5.1 and analyzed using SPSS version 20. Descriptive statistics, independent t-test, bivariable and multivariable logistic regression analysis were performed. A p-value of <0.05 was considered as statistically significant.

Result: From a total of 250 study participants, 125 were highly active antiretroviral therapy naïve and 125 were experienced. Of the total, 67.2% were females. The mean (±SD) age of the HAART naïve and experience individuals were 35.0(±9.5) and 45.0(±9.9) years, respectively. The overall prevalence of CKD among HIV infected study participants was 13.2%. The prevalence of CKD in HAART naïve and HAART experienced individuals was 15.2% and 11.2%, respectively. Age ≥ 50 years and CD4 count <200cells/mm^3 were statistically significant among HAART naïve participants. TDF regimen and CD4 count < 200 cells/mm^3 were statistically significant among HAART experienced participants.

Conclusion and recommendation: The prevalence of CKD was higher among HAART naïve than HAART experienced HIV infected individuals. CKD was associated with higher age, low CD4 count and TDF regimen users. A renal function assessment should be done before and during HAART to reduce renal dysfunction.

1. INTRODUCTION

1.1 Background

The human immunodeficiency virus (HIV) is a retrovirus that infects cells of the immune system, destroying or damaging their function. HIV 1 and 2 are the primary cause of acquired immunodeficiency syndrome (AIDS). HIV-1 is the major cause of AIDS in the world (1).

After global treatment goal was began in 2003, annual AIDS-related deaths have decreased by 43%. Global coverage of antiretroviral therapy (ART) reached 46% at the end of 2015. Eastern and Southern Africa are the most affected region by ADIS related death in the world. In these region ART coverage enlarged from 24% in 2010 to 54% in 2015, reaching a total of 10.3 million people (2).

Renal disorders in HIV patients is noticeable ranging from fluid and electrolyte imbalances to end stage renal disease (ESRD) regardless of HIV stages. Treatment-related factors, recurrent viremia, and traditional risk factors may be attributable for chronic kidney disease (CKD) (3).

Renal function impairment that results from HIV infection is called human immunodeficiency virus associated nephropathy (HIVAN) which is predominantly prevalent in black people specially sub-Sahara population (4, 5). Antiretroviral (ARV) drugs can also result renal damage which is presenting as acute kidney injury, tubular necrosis, kidney stones, or CKD (4, 6).

Renal toxicity associated with the use of nucleoside analogues is generally rare. Case reports have demonstrated that didanosine and lamivudine-stavudine treatment have been associated with tubular dysfunction (7, 8).

Tenofovir and cidofovir are acyclic nucleoside phosphonates that have been associated with renal tubular damage. The renal adverse effects may cause a variety of clinical presentations

varying from tubule cell death, such as acute tubular necrosis, to possibly reversible tubular dysfunction (9).

Tenofovirdisoproxil fumarate (TDF) is nucleotide analog reverse transcriptase inhibitor (NRTI), and may be associated with its tubular damage representing mitochondrial dysfunction (10).

TDF can decreased glomerular filtration rate, including Fanconi syndrome, also known as multiple tubular dysfunction syndrome, manifested as renal glucosuria, amino acids, urine, hypercalciuria, renal failure, and other renal toxicity, the renal toxicity has been widespread concern, now on the TDF in the HIV/AIDS (11).

HAART regimens containing the combination tenofovir/atazanavir is associated with low estimated glomerular filtration rate which leads to increase risk of CKD development (12).

Generally, renal system excrete many drug and their metabolites through the proximal tubule. So that drug related damage can be occurred because of the presence of high rate of blood flow with high level of toxin via proximal tubule (10).

The current study was intended to assess CKD and related factors both in HAART naïve and HAART experienced HIV infected patients at the University of Gondar Hospital, Northwest Ethiopia.

1.2. Statement of the problem

The introduction of highly active antiretroviral therapy has led to noticeable reduction in HIV-related mortality (13). However, renal disease is becoming an increasingly prevalent co-morbidity and mortality in patients with HIV infection. The increase in life expectancy following the introduction of HAART and the long term development of metabolic complications such as diabetes and dyslipidemia, hypertension, and vascular diseases can contribute to the increasing frequency in the recognition of renal impairment in HIV-infected patient (14).

A 4% to 17% prevalence of reduced kidney function in diverse HIV-infected populations was reported (15-17). Reports from different countries demonstrated that the occurrence of CKD with HIV infection was significantly high. For instance, 5.6% in Brazil, 18% in Switzerland, 27% in India, 12.3% in Iran, (18).

The contribution of HIV/AIDS to the prevalence of CKD in North-Central zone of Nigeria was as high as 47.6% (19). Using different criteria for diagnosis of CKD, different scholars reported a high burden of CKD in patients with HIV in different Africa countries; South Africa (6%), Nigeria (38%), Côte d'Ivoire (26%), Zambia (33.5%), Tanzania (28%), Uganda (20–48.5%), and Kenya (25%) (20).

The burden of CKD in HIV patients was reported in Southwest (18.2%) and Northwest (21.5% and 11.7%) of Ethiopia (21-23).

The presence of CKD in HIV patients will lead to death even if they take Antiretroviral therapy (24). Individuals with CKD are more likely to experience drug adverse effects with protease inhibitors (25). Renal disease in HIV-infected individuals, is a contributing factor for morbidity and mortality (26).

Risk factors associated with CKD in HIV-infected populations include aging, female gender, hypertension, diabetes mellitus, low CD4 cell count, and ART exposure (16, 27, 28).

2. LITERATURE REVIEW

At all stages of HIV infection, renal disorders may be evident, ranging from fluid and electrolyte imbalances to end stage renal disease (3). ARV drugs can result renal damage which is presenting as acute renal failure, tubular necrosis, kidney stones, or CKD (4, 6).

A retrospective observational analysis in a total of 5905 participants using the clinical database of a large center in Institute of Tropical Medicine in the urban area of Antwerp, Belgium showed that the prevalence of CKD among HIV infected subjects was found to be 3.0%. The development of CKD was associated with age above 50 years and lower CD4 cell counts (29).

According to a cross sectional study done in Brazil with 198 patients revealed that CKD was diagnosed in 8.4% of the population. The factors significantly associated with CKD were hypertension, time on HAART and tenofovir exposure (30).

A similar study done in a total of 322 Chinese HIV-infected participants indicated that the prevalence of CKD and proteinuria was 16.8%and 2.6%, respectively and 5.6% had GFR less than 60 ml/min/1.73 m^2. Older age and use of indinavir therapy were associated with development of CKD (16).

Likewise, another multicenter cross-sectional study done in Mainland, China in 538 HIV-infected ART-naïve patients revealed that the prevalence of CKD 16.1%. As stated by this study, 13.7% patients had proteinuria. According to this study older age, hypertension, and were significantly associated with CKD (31).

Alternative cross sectional study conducted in Kwara state, Nigeria with 183 treatment-naive HIV-infected patients revealed that 24% patients had CKD. The eGFR, 12% patients had stage 1, 63.9% stage 2, 7.1% stage 3, 14.8% stage 4, and 2.`2% stage 5 CKD (32).

In a similar cross-sectional study of 227 newly diagnosed, ARV naïve patients with HIV/AIDS carried out in Ilorin, Nigeria indicated that CKD was observed in 47.6% among

the patients and 16.7% of the controls. Forty one percent of the patients had dipstick proteinuria of ≥ 2 + (19).

A multicenter cross-sectional survey was carried out at Burundi in 300 patients revealed that CKD prevalence in patients was 45.7%. According to this study, 30.2% of whom being classified as stage 1, 13.5% as stage 2 and 2% as stage 3. No patient was classified as stage 4 or 5. Among CKD patients with urinary abnormality, proteinuria accounted for 6.1% and leukocyturia for 18.4%. Significant associations were found between leukocyturia, previous history of tuberculosis, low BMI and female gender (33).

According to cross-sectional observational study conducted in Nairobi, Kenya showed that evidence of CKD was seen in 88% of patients; 17.6% had eGFRCKD and 86.3% had albuminuria and no preselected risk factors were found to have significant association with presence of CKD (34).

A facility based comparative cross-sectional study conducted in 446 HIV positive individuals in Jimma, Ethiopia showed that the overall prevalence of renal function impairment was 18.2%. The prevalence of renal impairment in HAART naïve and HAART experienced persons was 28.7% and 7.6%, respectively. Age ≥ 50 years and advanced WHO stage were independent risk factors among HAART naïve participants. Female gender and age ≥50years were independent risk factors among HAART experienced participants. There was no difference between glucose level of HAART naive and HAART experienced participants (21).

A similar study was conducted among 307 HIV positive patients at Bahir Dar, Ethiopia showed that the prevalence of renal impairment in HAART naïve and on HAART individuals was 30.1% and 12.9% respectively. As stated by this study the average serum creatinine concentration and BUN were higher and mean creatinine clearance was lower in HAART naïve individuals compared with those individuals who were on HAART. It was also found that higher age, lower CD4 count, advanced in WHO clinical stage and being HAART naïve were associated with moderate to severe renal impairment. In HAART experienced individuals only low CD4 was found to be the independent risk factor (22).

A similar study was conducted in 275 study subjects in Gondar, Ethiopia indicated that the overall prevalence of CKD HAART treatment naive and on HAART treatment was 3.6% and 11.7%, respectively. According to this study a majority of the CKD patients were in stage 3 for patients on HAART treatment than HAART treatment naive. 3.1% of the patients had renal failure HAART treatment than HAART treatment naive. Being female was a risk factor for CKD (23).

Generally the prevalence of CKD in HAART naive and HAART treatment presence in different magnitude both in developed and developing countries. There may be different factors and different magnitudes based on different study design used, sample size, ethnicity, sex, age, definition of CKD, selection of formula for eGFR. Thus the aim of this study was to assess CKD and associated factors among HAART Naïve and HAART Experienced adult HIV positive individuals at the University of Gondar Hospital, Northwest Ethiopia.

3. SIGNIFICANCE OF THE STUDY

HIV affects all parts of the world, but sub-Saharan Africa is the highest epidemic area. Ethiopia currently has high numbers of people affected by the problem.

Chronic kidney disease becomes high and fast growing public health burden in HIV infected patients. Even though there is high disease burden of HIV in Ethiopia, we have limited data on HIV related kidney disease. Therefore, the purpose of this study is to assess chronic kidney disease among HIV infected patients attending at the University of Gondar Hospital. The findings will be supportive to early diagnosis and treatment of HIV related renal dysfunction. In addition, this study will give reliable information regarding associated factors which may increase the development of chronic kidney disease. It will be also helpful for clinicians to know associated factors to manage HIV/ADIS patients. This study may use as a reference for further studies.

4. OBJECTIVES OF THE STUDY

4.1. General objective

- To assess chronic kidney disease and associated factors among HAART naïve and HAART experienced HIV infected individuals at the University of Gondar Hospital, Northwest Ethiopia.

4.2. Specific objectives

- To determine the prevalence of CKD among HAART naïve and HAART experienced HIV infected individuals.
- To identify the associated factors of CKD among HAART naïve and HAART experienced HIV infected individuals.
- To compare mean of eGFR and creatinine level between HAART naïve and HAART experienced HIV infected individuals.

5. METHODS AND MATERIALS

5.1. Study area

The study was conducted at the University of Gondar Hospital ART clinic. The Hospital is located in North Gondar, Northwest Ethiopia around 747 km away from the capital city, Addis Ababa. The Hospital is one of the biggest Hospitals in north Gondar that provides health services and acts as the referral center for other district hospitals. It has over 400 beds including one ART clinic. The ART clinic gives service for a total of over 13,863 HIV infected individuals.

5.2. Study design and period

Hospital based comparative cross sectional study was conducted from March to May, 2017 to assess chronic kidney disease and associated factors among HAART naïve and HAART experienced HIV infected individuals attending at the University of Gondar Hospital.

5.3. Population

5.3.1. Source population

All HIV infected HAART naïve and HAART experienced individuals who had access to be served at the University of Gondar Hospital ART clinic.

5.3.2. Study population

All HIV infected HAART naïve and HAART experienced individuals who visited the hospital during the study period and fulfill the eligibility criteria.

5.4. Inclusion and exclusion criteria

5.4.1. Inclusion criteria

≥18 years old HIV infected HAART naïve and HAART experienced, Individuals ≥1 year since start ART.

5.4.2. Exclusion criteria

Severely ill patients (unable to speak, admitted patients), pregnant women, >65 years, and amputees were excluded from the study.

5.5. Study variable

5.5.1. Dependent variable

- ❖ Chronic kidney disease (CKD)

5.5.2. Independent variables

- ✓ Socio-demographic characteristics :- age, gender, religion, educational level, income,
- ✓ Co-morbidity:- Diabetes mellitus, Hypertension, acute kidney disease, kidney stone,
- ✓ HIV related characteristics:- Duration of HIV after diagnosis, ARV, CD4 count, Stage of HIV infection, regimen types,
- ✓ Anthropometric measurements:- weight, height, BMI,
- ✓ Laboratory tests: - Cr, Urea, Glucose, CrCl, eGFR, urine chemical tests (dipsticks).

5.6. Operational definitions

The eGFR was estimated using the Modification of Diet in Renal Disease (MDRD) Study equation: 186 × [serum creatinine ($\frac{\text{mg}}{\text{dl}}$)]$^{-1.154}$× $(age)^{-0.203}$ × (0.742 if female) x (1.210) by using eGFRonline calculator.

After calculating the eGFR, patients having CKD were classified into five stages of CKD according to the National Kidney Foundation – Kidney Disease Outcome Quality Initiative (KDOQI)classification system (35, 36)as follows:

- Stage 1, persistent proteinuria with eGFR ≥ 90 ml/min/1.73 m^2,
- Stage 2, persistent proteinuria with eGFR of 60-89.9 ml/min/1.73 m^2,
- Stage 3, eGFR 30-59.9 ml/min/1.73 $m^2$with or without proteinuria,
  - ✓ 3A (eGFR 45–59.9ml/min/1.73 m^2),
  - ✓ 3B (eGFR 30–44.9ml/min/1.73 m^2),
- Stage 4, eGFR 15-29.9 ml/min/1.73 $m^2$with or without proteinuria and

- Stage 5 (kidney failure), eGFR<15ml/min/1.73 m^2 with or without proteinuria.

Chronic kidney disease was defined as eGFR<60ml/min/1.73 m^2 with proteinuria.

HAART experienced persons who took HAART for more than 1 year which is composed of two NRTIs plus a NNRTI (37).

5.7. Sample size

The sample size was determined using double population proportion general formula by taking two different proportions of chronic renal disease (3.6%) for HAART naïve and (11.7%) for HAART experienced participants from the work done in Northwest of Ethiopia (23), taking a critical value at 95% confidence level, the level of significance 0.05 and Power (1-β) =90. By inserting these assumptions in to Epi-Info software the calculated sample size was 227, 113.5 for each group. By adding 10% compensation for non-respondents a total of 250 individuals, 125 for each group, were selected and convenient sampling technique was used.

$N=2 \times (p)(1-p)(z\beta + \frac{z\propto}{2})^2/(p1-p2)^2$

$=2(0.0765)\ (1\text{-}0.0765)\ (1.28\times1.96)^2\ /\ (0.036\text{-}0.117)^2$

$= 2\times \underline{(0.0765) \times (0.9235) \times (10.4976)}$

0.006561

$= 226.0728 \approx 227$

$= 227\times10\%$, Assuming 10% non-respondent rate

$= 22.7 \approx 23$, $=227+23$

N =250

Where

- n= minimum sample size
- p = best estimate of double population proportion, $p = p1 + p2 \therefore 2$
- P1= proportion of CKD for HAART naïve= 3.6%
- P2= proportion of CKD for HAART experienced= 11.7%
- Z_β= Power (1-β) =90% = 1.28
- $Z_{\alpha/2}$= level of significance 0.05 = 1.96

5.8. Data collection and laboratory methods

First, study participants were asked to fill the written consent form after pre-test was conducted in 5% of the study participants in Koladiba Hospital. Then Socio-demographic characteristics and clinical data were collected by trained nurses using semi-structured questionnaire. In addition, anthropometric and blood pressure measurement was taken by qualified personnel.

Five milliliter of venous blood sample was collected in gel containing serum separator test tubes and then serum was separated after the sample was clotted and centrifuged by trained laboratory technologists. Biochemical analysis was done using Mindray BS-200 chemistry analyzer (Shenzhen Mindray Bio-Medical Electronics Co. Ltd, China) for creatinine, and urea level determination. Fifty milliliter of freshly voided urine was collected by clean, dry container then urine dipstick was determine by using reagent strip with in 30 minute of collection according to manufacturer instruction.

5.9. Data management and quality Control

The questionnaire was pre-tested in Koladiba Hospital for its accuracy and consistency prior to actual data collection. Appropriate one day training was given for data collectors about the objectivity and relevance of the study, confidentiality issues, study participants' right, consenting, techniques of interview, and regarding laboratory test procedures and their quality control. Socio-demographic and clinical data were collected by two trained nurses under the supervision of investigators. The biochemical tests were performed by two senior medical laboratory technologists under the supervision of principal investigator. Furthermore, the investigator was closely followed up and frequently check the data collection process to ensure the completeness and consistency of the collected data and also give feedback on a daily basis to the data collectors. Completion, accuracy, and clarity of the collected data was checked carefully on a regularly basis.

The sample was processed within 1 hour of specimen collection in Clinical Chemistry Laboratory and processed based on the manufacturer's manual. Normal and abnormal controls were run daily in order to check the optimal reactivity of the reagent and

functionality of the machine. The values obtained for controls must be within ± 2SD of the given range. Coefficient of variation must be <5%. Moreover, to maintain the quality of the result, pre-analytical, analytical and post analytical pre-cautions of quality depending on the stated SOP was considered and quality control results were interpreted using Levy Jenny chart and appropriate remedies was taken in case of unacceptable quality control results.

5.10. Data analysis

The Data was checked, cleaned, sorted, and categorized manually. Then, the data was entered to Epi info version 3.5.1 and then transferred to SPSS version 20 for analysis. Descriptive statistics, independent t test, bivariable and multivariable logistic regression analyses was performed. The multivariable model incorporated a backward and stepwise elimination method using variables with a P-value of <0.25 from the bivariable analysis. OR with95%CI were also obtained. All analyses were performed using SPSS version 20.0.A p value < 0.05 was considered to be statistically significant.

5.11. Ethical consideration

Before starting the study, ethical clearance was obtained from Research and Ethical Review Committee of School of Biomedical and Laboratory Sciences, College of Medicine and Health Sciences, University of Gondar and the acquiescence was also obtained from the study institutions (University of Gondar Hospital). Full clarification about the purpose of the study was made to the Authorized persons of the Hospital. Permission letter was also taken from the clinical director of the Hospital and head of the ART clinic. To ensure confidentiality of the study participant's information, anonymous typing was applied so that the name of the participant and any identifier of participants was not written on the questionnaire. Data was collected after full written consent obtained from each participant. The data were not used for another purpose.

5.12. Result dissemination

This study on completion could serve as a reference material for researchers, experts and policy makers for intervention. To reach these bodies a copy of completed paper were submitted to the University of Gondar, College of Medicine and Health Sciences, School of Biomedical and Laboratory Sciences, Department of Clinical Chemistry and University of Gondar Hospital.

The result will also be disseminated through publication in peer reviewed local and international journal and through presenting it in related annual scientific meetings, conferences and seminars.

6. RESULT

6.1. Socio demographic characteristics of study participants

From a total of 250 HIV infected study participants, 168 (67.2%) were females. The mean (±SD) age of the study participants was 35 (±10) years and 45 (±10) years for HAART naïve and experienced patients, respectively. Majority (85.6%) were urban inhabitant, 56.4% were married and 28% of them were unable to read and write. During the study, 18.8% and 11.2% of study participants were alcohol drinkers and cigarette smokers, respectively (Table 1).

Table 1: Socio-demographic characteristics of HIV infected participants attending at the University of Gondar Hospital, Gondar, Northwest Ethiopia, 2017 (N=250).

Variables		HAART naive No (%)	On HAART No (%)	Total No (%)
Age (years)	18-28	37(29.6)	1(0.8)	38(15.2)
	29-38	47(37.6)	36(28.8)	83(33.2)
	39-48	29(23.2)	46(36.8)	75(30.0)
	≥40	12(9.6)	42(33.6)	54(21.6)
Sex	Male	47(37.6)	35(28.0)	82(32.8)
	Female	78(62.4)	90(72.0)	168(67.2)
Religion	Christian	108(86.4)	109(87.2)	217(86.8)
	Muslim	17(13.6)	16(12.8)	33(13.2)
Ethnicity	Amhara	116(92.8)	116(92.8)	232(92.8)
	Tigre	9(7.2)	9(7.2)	18(7.2)
Residency	Urban	106(84.8)	108(86.4)	214(85.6)
	Rural	19(15.2)	17(13.6)	36(14.4)
Marital status	Single	15(12.0)	11(8.8)	26(10.4)
	Married	73(58.4)	68(54.4)	141(56.4)
	Windowed	11(8.8)	31(24.8)	42(16.8)
	Divorced	26(20.8)	15(12.0)	41(16.4)
Educational level	Unable to read and write	30(24.0)	40(32.0)	70(28)
	Primary	47(37.6)	37(29.6)	84(33.6)
	Secondary	32(25.6)	31(24.8)	63(25.2)
	Higher	16(12.8)	17(13.6)	33(13.2)
Occupation	Unemployed	53(42.4)	39(31.2)	92(36.8)
	Self employed	50(40.0)	58(46.4)	108(43.2)
	Governmental	22(17.6)	28(22.4)	50(20)
Smoking habit	No	107(85.6)	115(92.0)	222(88.8)
	Yes	18(14.4)	10(8.0)	28(11.2)
Alcohol habit	No	81(64.8)	122(97.6)	203(81.2)
	Yes	44(35.2)	3(2.4)	47(18.8)

Note: - HAART = Highly Active Anti-Retroviral Therapy, HIV= Human Immunodeficiency Virus

6.2. Clinical related information among HIV infected study participant

About 51.2% of HIV infected study participants were found in WHO stage II. Less than half (45.6%) of HAART naïve and 67.2% of HAART experienced study participants were found in WHO stage III and stage II respectively. Almost all (98.4%) of HAART experienced study participants were receiving first line ART regimen. More than half (54.4%) of HAART experienced study participants were non TDF regimen users. Majority of (94.4%) HAART naïve study participants were <5 years since diagnosis of HIV. On the other hand, over half (52.8%) of HAART experienced participants were between 5-10 years since after diagnosis of HIV (Table 2).

Table 2: HIV related information among HIV infected participants at the University of Gondar Hospital, Gondar, Northwest Ethiopia, 2017 (N=250).

Variables		HAART naïve No (%)	On HAART No (%)	Total No (%)
Time since diagnosis of HIV (years)	<5	118(94.4)	40(32.0)	158(63.2)
	5-10	7(5.6)	66(52.8)	73(29.2)
	>10	0(0)	19(15.2)	19(7.6)
WHO HIV stage	I	13(10.4)	17(13.6)	30(12)
	II	44(35.2)	84(67.2)	128(51.2)
	III	57(45.6)	23(18.4)	80(32)
	IV	10(8.0)	1(0.8)	12(4.8)
	V	1(0.8)	0(0)	1(0.4)
CD4+ (cells/mm^3)	<200	58(46.4)	28(22.4)	86(34.4)
	200-350	42(33.6)	38(30.4)	80(32)
	>350	25(20.0)	59(47.2)	84(33.6)
Time since on ART (years)	<5		48(38.4)	
	5-10		35(28.0)	
	>10		42(33.6)	
ART regimen	First		123(98.4)	
	Second		2(1.6)	
First line regimen types	1c		47(37.6)	
	1d		20(16.8)	
	1e		48(38.4)	
	1f		5(6.4)	

Note:-1c= AZT+3TC+NVP, 1d= AZT+3TC+EFV, 1e= TDF+3TC+EFV, 1f= TDF+3TC+ NVP= 3TC= lamivudine, AZT=zidovudine, NVP= nevirapine, EFV= efavirenz, TDF= Tenofovir Disoproxil Fumarate, CD4=Cluster of Differentiation4, WHO=World Health Organization, HIV= Human Immunodeficiency Virus, ART=Anti-Retroviral Therapy, HAART =Highly Active Anti-Retroviral Therapy.

History of hypertension and diabetes mellitus were reported in 12(4.8%) and 11(4.4%) of the participants respectively. About 55.6% of study participants had a normal BMI (Table 3).

Table 3; Preselected risk factors among HIV infected study participants at the University of Gondar Hospital, Gondar, Northwest Ethiopia, 2017 (N=250).

Variables		HAART naïve No (%)	On HAART No (%)	Total No (%)
BMI (kg/m^2)	<18.5	37(29.6)	19(15.2)	56(22.4)
	≥18.5-24.9	61(48.8)	78(62.4)	139(55.6)
	≥25-29.9	25(20.0)	23(18.4)	48(19.2)
	≥30	2(1.6)	5(4.0)	7(2.8)
Diabetes mellitus	No	117(93.6)	122(97.6)	239(95.6)
	Yes	8(6.4)	3(2.4)	11(4.4)
Blood pressure	No	115(92.0)	123(98.4)	238(95.2)
	Yes	10(8.0)	2(1.6)	12(4.8)
Acute kidney injury	No	99(79.2)	97(77.6)	196(78.4)
	Yes	26(20.8)	28(22.4)	54(21.6)
Kidney stone history	No	114(91.2)	118(94.4)	232(92.8)
	Yes	11(8.8)	7(5.6)	18(7.2)

Note: - Body Mass Index (BMI), Acute Kidney Injury (AKI), HAART =Highly Active Anti-Retroviral Therapy, HIV =Human Immunodeficiency Virus

6.3. Renal function tests among HIV infected study participant

The mean (±SD) of eGFR (MDRD) was 96.0 (± 44.8) ml/min/1.73 m^2 and 85.3(±20.0) ml/min/1.73 m^2 for HAART naïve study participants and for those who were HAART experienced, respectively. The mean (±SD) Cr level was 1.1 (±0.7mg/dl) for HAART naïve study participants and 0.98 (± 0.2 mg/dl) for those who were HAART experienced. Proteinuria 1+ and above was found in 34.4% and 47.2% of HAART naïve and HAART experienced study participants, respectively (Table 4).

Table 4:Mean of biochemical parameters among HIV infected participants at the University of Gondar Hospital, Gondar, Northwest Ethiopia, 2017 (N=250).

Parameters		HAART naïve Mean ±SD	On HAART Mean ±SD	Total Mean ±SD
Cr (mg/dl)ℸ		1.1±0.7	0.98±0.2	1.04±0.45
Urea (mg/dl)		18.5±15.0	19.4±10.1	18.95±12.55
CrCl C-G (ml/min)		73.8±33.2	67.8±19.2	70.8±26.2
eGFR(MDRD) (ml/min/1.73m^2)		96.0±44.8	85.3±20.0	90.65±32.4
CD4 (cells/mm^3)		245.9±163.5	391.3±209.5	318.6±186.5
Proteinuria	Neg.	82(65.6)	66(52.8)	148(59.2)
	1+$^{\kappa}$	32(25.6)	39(31.2)	71(28.4)
	2+	11(8.8)	20(16.0)	31(12.4)
Hematuria	Neg.	101(80.8)	104(83.2)	205(82.0)
	1+	14(11.2)	16(12.8)	30(12.0)
	2+	10(8.0)	5(4.0)	15(6.0)
Leucocytouria	Neg.	96(76.8)	105(84.0)	201(80.4)
	1+	19(15.2)	16(12.8)	35(14.0)
	2+	10(8.0)	4(3.2)	14(5.6)

Note: -ℸ=Based on single measurement, κ=30mg/dl on urine dipstick, HAART =Highly Active Anti-Retroviral Therapy, HIV =Human Immunodeficiency Virus, Cr =Creatinine, CrCl =Creatinine Clearance, eGFR =estimated Glomerular Filtration Rate, MDRD= Modification of Diet for Renal Disease, CD4= Cluster of Differentiation 4, Neg= Negative.

6.4. Comparison of biochemical and other variables between HAART naïve and experienced

The mean (±SD) of Cr of HAART naïve and HAART experienced study participants was 1.1±0.7 and 0.98±0.2, respectively (p =0.01). HAART naïve study participants had a significantly higher mean eGFR compared to the HAART experienced (96.0±44.8 ml/min/1.7m^2) vs (85.3±20.0 ml/min/1.73 m^2); (p =0.01). Urea level, and CrCl, showed no significant differences between HAART naïve and HAART experienced study participants. The mean (±SD) of CrCl in HAART naive study participants was 73.8±33.2 and the mean (±SD) of CrCl level among HAART experienced was 67.8±19.2 (p=0.08). The mean (±SD) BMI of the study participants was 21.0 (±4.0) and 22.0 (±4.0) for HAART naïve and HAART experienced study participants respectively; (p = 0.03). The mean (±SD) time since HIV diagnosis was significantly different between HAART naïve and HAART experienced participants (p value = 0.01) (Table 5).

Table 5: Comparison of biochemical and other variables between HAART naive and HAART experienced individuals at the University of Gondar Hospital, Gondar, Northwest Ethiopia,2017 (N = 250).

Parameters	HAART naïve Mean ± SD	On HAART Mean ± SD	p-value
Cr (mg/dl)	1.1±0.7	0.98±0.2	0.01
Urea (mg/dl)	18.5±15.0	19.4±10.1	0.60*
CrCl (C-G) (min/ml)	73.8±33.2	67.8±19.2	0.08*
eGFR (MDRD) (ml/min/1.73 m^2)	96.0±44.8	85.3±20.0	0.01
CD4+ (cells/mm^3)	245.9±163.5	391.3±209.5	0.01
BMI (kg/m^2)	21±4	22±4	0.03
Time since HIV diagnosis (years)	2.24±1.7	7.4±4.2	0.01

Note: - *not significant, Cr= Creatinine, CrCl =Creatinine Clearance, eGFR =estimated Glomerular Filtration Rate, MDRD =Modification of Diet and Renal Disease, CD4 =Cluster of Differentiation 4, BMI =Body Mass Index, HIV =Human Immunodeficiency Virus, HAART =Highly Active Anti-Retroviral Therapy, SD =Standard Deviation

6.5. Prevalence of CKD among HIV infected study participants

The overall prevalence of CKD among HIV infected participants was 13.2% (95%CI 9.2, 17.6). The prevalence of CKD among HAART naïve study participants was 15.2% (95%CI 9.6, 20.8), whereas among HAART experienced study participants was 11.2% (95%CI 5.6, 17.6), p value 0.5. A majority of the CKD cases were observed in CKD stage 2; 28.8% HAART naïve and 33.6% HAART experienced. About 34.4% of HAART naïve study participants showed urine dipstick proteinuria, whereas less than half (47.2%) of HAART experienced study participants showed urine dipstick proteinuria (Table 6).

Table 6: Prevalence of CKD based on MDRD among HIV infected participants at University of Gondar Hospital, Gondar, Northwest Ethiopia, 2017 (N = 250).

Variables with descriptions			HAART naïve	On HAART	Total
			No (%)	No (%)	No (%)
CKD	<60	Yes	19(15.2)	14(11.2)	33(13.2)
	≥60	No	106(84.6)	111(88.8)	217(86.8)
Stages	1	≥ 90	10(8)	12(9.6)	22(8.8)
	2	60-89	36(28.8)	42(33.6)	78(31.2)
	3	30-59.9	18(14.4)	14(11.2)	32(12.8)
	4	15-29	0(0)	0(0)	0(0)
	5	< 15	1(0.8)	0(0)	1(0.4)
Proteinuria		No	82(65.6)	66(52.8)	148(59.2)
		Yes	43(34.4)	59(47.2)	102(40.8)

Note: -Proteinuria ≥ 1+, CKD=Chronic Kidney Disease, MDRD=Modification of Diet and Renal Disease, HAART =Highly Active Anti-Retroviral Therapy, HIV =Human Immunodeficiency Virus

6.6. Factors Associated with CKD among HIV infected study participants

Older age ≥50 years (AOR=8.9, 95%CI; 1.2, 68.4) and low CD4 count (<200cells/mm^3) (AOR=7.6, 95%CI; 1.9, 30.2) were independently associated factors for CKD among HAART naïve individuals (Table 7).

Table 7: Factors associated with CKD among HAART naive HIV infected participants at the University of Gondar Hospital, Gondar, Northwest Ethiopia, 2017 (N=125).

Variables		CKD <60	CKD ≥60	AOR(95%CI)	COR(95%CI)	P-value
		Yes	No			
Age (years)	<50$^+$	16	103	1		
	≥50	3	3	6.4(1.3-37.5)	8.9(1.2-68.4)	0.03*
Acute kidney injury	No$^+$	13	86	1		
	Yes	6	20	2(0.7-6)	1.9(0.5-7.6)	0.37
Kidney stone	No$^+$	14	100	1		
	Yes	6	5	8.6(2-22)	3.6(0.8-15.1)	0.08
CD4 (cells/mm^3)	<200	16	42	8.1(2.2-29.6)	7.6(1.9-30.2)	0.004**
	≥200	3	64	1		

Note: - += Reference, ***** = Significant, ****** = highly significant, CD4 = cluster of differentiation 4, CKD =Chronic Kidney Disease, Backward stepwise multiple logistic regression was used to assess the independent effect of explanatory variables

On the other hand, TDF based ART regimen (AOR= 6.0; 95%Cl 1.3-29.4) and CD4 count < 200 cells/mm^3 (AOR=18.5; 95%Cl 4.1-83.2) were independently associated with CKD among HAART experienced study participants (Table 8).

Table 8: Factors associated with CKD among HAART experienced at University of Gondar Hospital, Gondar, Northwest Ethiopia, 2017 (N=125).

Factors		CKD <60	CKD ≥60	COR(95%CI)	AOR(95%CI)	P value
		Yes	No			
Age (years)	<50+	5	85	1		
	≥50	9	26	5.8(1.8-19.1)	2.7(0.63-11.7)	0.18
Diabetes Mellitus	No+	12	110	1		
	Yes	2	1	18.3(1.5-217.4)	9.1(0.7-125.7)	0.09
First line regimen	Non TDF+	3	64	1		
	TDF	10	46	4.6(1.2-17.8)	6.0(1.3-29.4)	0.025**
CD4 (cells/mm^3)	<200	10	18	12.9(3.6-45.7)	18.5(4.1-83.2)	0.000**
	≥200+	4	93	1		

Note: - + = Reference, ** = highly significant, TDF = Tenofovir Disoproxil Fumarate, CD4 = Cluster of Differentiation 4, COR=Crude Odds Ratio, AOR = Adjusted Odds Ratio, CKD =Chronic Kidney Disease, Backward stepwise multiple logistic regression was used to assess the independent effect of explanatory variables.

Both bivariable and multivariable logistic regression analysis showed that older age (≥50 years), low CD4 count (<200cells/mm^3) and TDF based ART regimen had statistically significant and independently association with CKD among HIV infected study participants. Low CD4 count (<200cells/mm^3) was significantly associated with CKD among HIV infected study participants (p-value <0.01). CKD in HIV infected study participants with older age ≥50 years was found to be 6 times more likely to develop CKD than those < 50 years age (AOR=6; 95%CI 2, 12). HIV infected study participants who taken TDF based ART regimen were 6 times more likely to develop CKD compared to those non TDF based ART regimen users(AOR=6; 95%CI 2, 17). Among HIV infected individuals those who had CD4 count <200 cells/mm^3 were 9 times more likely to develop CKD than CD4 count ≥200 cells/mm^3(AOR=9; 95%CI 4, 27) (Table 9).

Table 9: Factors associated with CKD among HIV infected participants at the University of Gondar Hospital, Gondar, Northwest Ethiopia, 2017 (N=250).

Factors		CKD<60	CKD≥60	COR(95%CI)	AOR(95%CI)	P value
		Yes	No			
Age	<50$^+$	21	187	1		
	≥50	13	29	4(2-8)	6.4(1.9-12.3)	0.028*
Diabetes mellitus	No$^+$	30	209	1		
	Yes	4	7	4.1(1.2-15.0)	9.7(0.6-152.9)	0.10
Kidney stone	No$^+$	28	204	1		
	Yes	6	12	3.6(1.3-10.9)	7.6(0.6-97.0)	0.11
First line regimen	Non TDF$^+$	4	63	1		
	TDF	10	46	3.4(2.1-12)	6.3(2.7-17)	0.027*
Proteinuria	No$^+$	10	115	1		
	Yes	24	101	2.7(1.2-5.7)	0.2(0.05-1.0)	0.059
CD4 (cells/mm^3)	<200	27	59	10(4-24)	9(4-27)	0.01**
	≥200$^+$	7	157	1		

Note: - + = Reference, * = significant, ** = highly significant, TDF = Tenofovir Disoproxil Fumarate, CD4 = Cluster of Differentiation 4, COR=Crude Odds Ratio, AOR = adjusted odds ratio, Backward stepwise multiple logistic regression was used to assess the independent effect of explanatory variables.

7. DISCUSSION

Renal disease has been documented as a common and closely associated complication of HIV infection (38). HIV infected individuals can have many adverse factors which can affect the kidney arise from direct effects of HIV such as immune complex disease and HIVAN or indirectly from opportunistic infection and drugs (34).

The prevalence of CKD in all HIV infected study participants in this study based on eGFR using the MDRD equation was 13.2% (95%CI 9.2, 17.6). This result is in line with study done in China (16.8%) (16) but lower than study done in northwest of Ethiopia (21.5%) (22). The reason for this could be sample size difference and use of C-G formula for eGFR calculation. However, the prevalence of CKD according to current study was higher than studies done in Belgium (29) (3%), it was used large sample size with retrospective observational analysis but not used proteinuria for definition of CKD, Brazil (30) (8.4%), even though, current study similar by sample size with study in Brazil (255) obesity, malnutrition and history of kidney disease were excluded by study done in Brazil, Burundi (33) (2%), multicenter cross sectional study design and persistence measure for 3 months for Cr level and proteinuria was used by study done in Burundi, sub-Sahara Africa (39) (7%), used randomized control trial.

Therefore, the observed difference may be due to the difference in geographical variation, study design, sample size, definition of CKD, exclusion criteria, and measurement of proteinuria.

The prevalence of CKD among HAART naïve study participants was 15.2% (95%CI 9.6, 20.8). It is consistence with study done in Mainland China (16.1%) (31) and in Kenya (11.5%) (40) but much lower than study done in Kwara state, Nigeria (47.6%) (32) and Malawi (21%) (41). The reason for this variation could be those studies used different geographical distribution, and sample size; Nigeria (183), Malawi (526). Moreover, report from Malawian used C–G equation for estimated GFR.

Even though, there was difference in sample size (163 vs 125) used and study design (case-control vs cross-sectional) between study done in Ghana (9.9%) and the current study 11.2%

(95%CI 5.6, 17.6), the prevalence of CKD among HAART experience study participants was concordant with study done in Ghana (42). On the other hand, this study was lower as compared to studies done in Nigeria (23.8%) (43), and in Tanzania (25%) (44).The use of C-G formula to estimate GFR in Nigeria and Tanzania; furthermore, Nigerian study used retrospective study design and the number of study subjects may contribute to the observed differences.

In this study prevalence of proteinuria was (40.8%), (34.4%), and (47.2%) among total study participants, HAART naïve, and HAART experienced respectively. This study demonstrates high proteinuria when compared with studies done in Mainland China (13.7%) (31), India (21%) (45), Malawi (23.3%) (41), Burundi (6.1%) (33), Kenya (12%) (34), northwest Ethiopia (Bahir Dar) (17.9%) (22). On the other hand, a similar result was observed in Nigeria (41.4%) (19). Sample size, Geographical variation and the use of difference measuring technique of proteinuria may contribute for the observed difference. At normal physiological condition, small amounts of proteins are seen in the urine since its plasma concentration is low. When the glomerular filtration barrier is compromised by disease, an increased amount of plasma proteins is allowed to pass into the ultra-filtrate, and glomerular proteinuria develops (46).

Owing to the fact that renal function is known to decline with incline of age, older age is a proven risk factor for a decline in CrCl (47). According to this study the observed CKD in overall HIV infected study participants, and HAART naïve study participants with age ≥50 years were more likely to develop CKD than those <50 years age. This result consistence with studies done in Belgium, China and Southwest Ethiopia (16, 21, 29).

The result found from this study showed that low CD4 count (<200 cells/mm^3) was significantly associated with CKD among overall HIV infected, HAART naïve, and HAART experienced. A low CD4 count (<200cells/mm^3) was more likely to develop CKD among overall HIV infected, HAART naïve, and HAART experienced study participants. This result is in line with a study done in China (16), Nigeria (43), Zambia (48), and southwest (16) and northwest Ethiopia (22). In this study the association of CKD with low CD4 cell count may

suggest that HIVAN is a contributory cause of CKD in study participants since CD4 cell count is used as a surrogate marker of immunological status in HIV infected patients (4).

This study demonstrated the significant association between CKD with TDF based ART regimen among HAART experienced study participants. Being TDF based ART regimen user was more likely to develop CKD than non TDF user. Regarding TDF regimen users associate with CKD, this is in agreement with findings of studies conducted in New York (51), France (52), Netherlands (49), Denmark (12), Japan (50), Brazil (30), and South Africa (48). The reason for this association could be the mechanism by which tenofovir causes renal toxicity seems to be related to drug accumulation within proximal renal tubules, leading to mitochondrial DNA injury, depletion and dysfunction of the oxidative respiratory chain in proximal tubular epithelial cells. Therefore, this leads to a depletion of intracellular ATP, which limits the proximal tubule's ability to reabsorb electrolytes and low molecular weight proteins (10, 48, 49).

Generally, the current study indicated that older age (≥50 years), TDF based regimen, and low CD4 count (<200cells/mm^3) were identified as the predictors of CKD in overall HIV infected study participants. In addition, older age (≥50 years), and low CD4 count (<200cells/mm^3) were demonstrated as the predictors of CKD in HAART naive study participants. Moreover, TDF based regimen users, and low CD4 count were found to be the predictors of CKD in HAART experienced study participants.

8. LIMITATION AND STRENGTH OF THE STUDY

In this study creatinine level and proteinuria was measured once so consecutive results could not be determined which may be challenging to estimate the presence of CKD due to the possibility of reversal causes. As the result of cross-sectional nature of this study, preventing assessment of whether risk factors caused or resulted from decreased renal function. However, being prospective study, comparison of HAART status, assessment of associated factors for CKD could be considered as the strength of this study.

9. CONCLUSION

The overall prevalence of CKD and proteinuria found in this study was high. The results of this study indicated that HIV infected adults have high prevalence of CKD among HAART naïve than those HAART experienced at the University of Gondar Hospital. The current study revealed that a majority of the CKD cases were observed in CKD stage 2; 28.8% HAART naïve and 33.6% HAART experienced. Therefore, this study concluded that CKD is common in HIV patients. In addition, the current study revealed that those patients who are older (≥50 years), have a history of TDF regimen therapy, and low CD4 count (<200 cells/mm^3) are factors which can predict the likelihood of developing CKD in HIV infected participants.

10. RECOMMENDATIONS

- A close monitoring of renal function should be done before and during HAART.
- Special focus should be given for HIV infected individuals who develop any stages of chronic kidney disease in order to get an early treatment before reaching end stage renal disease.
- More strong longitudinal studies should be done to determine risk factors of CKD in HIV infected individuals.
- A result of this study showed that the prevalence of CKD among HIV infected individuals was high, so that further studies with large sample size and different study design are necessary. Findings from all such studies can be useful for better health care planning and delivery.

11. REFERENCES

1. Sharp PM, Hahn BH. Origins of HIV and the AIDS pandemic. *Cold Spring Harbor perspectives in medicine*. 2011;1(1):a006841.
2. UNAIDS. GLOBAL AIDS UPDATE 2016 [Available from: data.unaids.org/Publications/IRC-pub01/jc267-stratpla.
3. Okuonghae P, Olisekodiaka M, Onuegbu J, Amara A, Aberare L, Mukoro N, et al. Evaluation of renal function in HIV patients on antiretroviral therapy. *Adv Lab Med Int*. 2011;1:25-31.
4. Okafor U, Unuigbe E, Ojogwu L, Oviasu E, Wokoma F. Renal disease in HIV infected patients at University of Benin Teaching Hospital in Nigeria. *African health sciences*. 2011;11(3):28-33.
5. Avila-Casado MC, Fortoul TI, Chugh SS. HIV-associated nephropathy: experimental models. Experimental Models for Renal Diseases. 169: Karger Publishers; 2011. p. 270-85.
6. Röling J, Schmid H, Fischereder M, Draenert R, Goebel F. HIV-Associated Renal Diseases and Highly Active Antiretroviral Therapy—Induced Nephropathy. *Clinical infectious diseases*. 2006;42(10):1488-95.
7. Izzedine H, Launay-Vacher V, Deray G. Fanconi syndrome associated with didanosine therapy. *Aids*. 2005;19(8):844-5.
8. Morris AA, Baudouin SV, Snow MH. Renal tubular acidosis and hypophosphataemia after treatment with nucleoside reverse transcriptase inhibitors. *Aids*. 2001;15(1):140-1.
9. Daugas E, Rougier J-P, Hill G. HAART-related nephropathies in HIV-infected patients. *Kidney international*. 2005;67(2):393-403.
10. Izzedine H, Harris M, Perazella MA. The nephrotoxic effects of HAART. *Nature Reviews Nephrology*. 2009;5(10):563-73.
11. Geng T, Li T. Renal toxicity of tenofovir in HIV/AIDS patients. *Zhonghua nei ke za zhi*. 2016;55(9):737.
12. Rasch MG, Engsig FN, Feldt-Rasmussen B, Kirk O, Kronborg G, Pedersen C, et al. Renal function and incidence of chronic kidney disease in HIV patients: a Danish cohort study. *Scandinavian journal of infectious diseases*. 2012;44(9):689-96.

13. Modrich L, Scherzer R, Zolopa A, Rimland D, Lewis CE, Bacchetti P, et al. Association of HIV infection, demographic and cardiovascular risk factors with all-cause mortality in the recent HAART era. *Journal of acquired immune deficiency syndromes (1999)*. 2010;53(1):102.
14. Crum NF, Riffenburgh RH, Wegner S, Agan BK, Tasker SA, Spooner KM, et al. Comparisons of causes of death and mortality rates among HIV-infected persons: analysis of the pre-, early, and late HAART (highly active antiretroviral therapy) eras. *JAIDS Journal of Acquired Immune Deficiency Syndromes*. 2006;41(2):194-200.
15. Duval X, Journot V, Leport C, Chêne G, Dupon M, Cuzin L, et al. Incidence of and Risk Factors for Adverse Drug Reactions in a Prospective Cohort of HIV-Infected Adults Initiating Protease Inhibitor—Containing Therapy. *Clinical infectious diseases*. 2004;39(2):248-55.
16. Cheung CY, Wong KM, Lee MP, Liu YL, Kwok H, Chung R, et al. Prevalence of chronic kidney disease in Chinese HIV-infected patients. *Nephrology Dialysis Transplantation*. 2007;22(11):3186-90.
17. Lucas GM, Mehta SH, Atta MG, Kirk GD, Galai N, Vlahov D, et al. End-stage renal disease and chronic kidney disease in a cohort of African-American HIV-infected and at-risk HIV-seronegative participants followed between 1988 and 2004. *Aids*. 2007;21(18):2435-43.
18. Naicker S, Fabian J. Risk factors for the development of chronic kidney disease with HIV/AIDS. *Clinical nephrology*. 2010;74:S51-6.
19. Ayokunle DS, Olusegun OT, Ademola A, Adindu C, Olaitan RM, Oladimeji AA. Prevalence of Chronic Kidney Disease in newly diagnosed patients with Human immunodeficiency virus in Ilorin, Nigeria. *Jornal Brasileiro de Nefrologia*. 2015;37(2):177-84.
20. Fabian J, Naicker S. HIV and kidney disease in sub-Saharan Africa. *Nature Reviews Nephrology*. 2009;5(10):591-8.
21. Mekuria Y, Yilma D, Mekonnen Z, Kassa T, Gedefaw L. Renal Function Impairment and Associated Factors among HAART Naïve and Experienced Adult HIV Positive Individuals in Southwest Ethiopia: A Comparative Cross Sectional Study. *PloS one*. 2016;11(8):e0161180.
22. Kahsu G, Birhan W, Addis Z, Dagnew M, Abera B. Renal Function Impairment and Associated Risk Factors among Human Immunodeficiency Virus Positive Individuals at

Flege Hiwot Referral Hospital, Northwest Ethiopia. *Journal of Interdisciplinary Histopathology*. 2013;1(5):252-60.

23. Baynes HW, Tegene B, Gebremichael M, Birhane G, Kedir W, Biadgo B. Assessment of the effect of antiretroviral therapy on renal and liver functions among HIV-infected patients: a retrospective study. *HIV/AIDS (Auckland, NZ)*. 2017;9:1.
24. Calza L. Renal toxicity associated with antiretroviral therapy. *HIV clinical trials*. 2012;13(4):189-211.
25. Szczech LA, Gupta SK, Habash R, Guasch A, Kalayjian R, Appel R, et al. The clinical epidemiology and course of the spectrum of renal diseases associated with HIV infection. *Kidney international*. 2004;66(3):1145-52.
26. Szczech LA, Hoover DR, Feldman JG, Cohen MH, Gange SJ, Goozé L, et al. Association between renal disease and outcomes among HIV-infected women receiving or not receiving antiretroviral therapy. *Clinical infectious diseases*. 2004;39(8):1199-206.
27. Mocroft A, Kirk O, Gatell J, Reiss P, Gargalianos P, Zilmer K, et al. Chronic renal failure among HIV-1-infected patients. *Aids*. 2007;21(9):1119-27.
28. Ando M YN. Epidemiology, clinical characteristics, and management of chronic kidney disease in human immunodeficiency virus-infected patients. *World journal of nephrology*. 2015;4(3):388-95.
29. Colson A, Florence E, Augustijn H, Verpooten G, Lynen L, Gheuens E. Prevalence of chronic renal failure stage 3 or more in HIV-infected patients in Antwerp: an observational study. *Acta Clinica Belgica*. 2010;65(6):392-8.
30. Menezes AM, Torelly Jr J, Real L, Bay M, Poeta J, Sprinz E. Prevalence and risk factors associated to chronic kidney disease in HIV-infected patients on HAART and undetectable viral load in Brazil. *PloS one*. 2011;6(10):e26042.
31. Cao Y, Gong M, Han Y, Xie J, Li X, Zhang L, et al. Prevalence and risk factors for chronic kidney disease among HIV- infected antiretroviral therapy- naïve patients in Mainland China: A multicenter cross- sectional study. *Nephrology*. 2013;18(4):307-12.
32. Adedeji TA, Adedeji NO, Adebisi SA, Idowu AA, Fawale MB, Jimoh KA. Prevalence and Pattern of Chronic Kidney Disease in Antiretroviral-Naive Patients with HIV/AIDS. *Journal of the International Association of Providers of AIDS Care (JIAPAC)*. 2015;14(5):434-40.

33. Cailhol J, Nkurunziza B, Izzedine H, Nindagiye E, Munyana L, Baramperanye E, et al. Prevalence of chronic kidney disease among people living with HIV/AIDS in Burundi: a cross-sectional study. *BMC nephrology*. 2011;12(1):40.
34. Kairu BN. Prevalence Of Chronic Kidney Disease Among Ambulatory Human Immunodeficiency Virus/acquired Immunodeficiency Syndrome Patients On Antiretroviral Therapy At The Kenyatta National Hospital: university of Nairobi; 2015.
35. Levey AS. A decade after the KDOQI CKD guidelines. WB Saunders; 2012.
36. Hogg RJ, Furth S, Lemley KV, Portman R, Schwartz GJ, Coresh J, et al. National Kidney Foundation's Kidney Disease Outcomes Quality Initiative clinical practice guidelines for chronic kidney disease in children and adolescents: evaluation, classification, and stratification. *Pediatrics*. 2003;111(6):1416-21.
37. Denue A, Muazu J, Gashau W, Nkami D, Ajayi NA. Effects of highly active antiretroviral therapy (HAART) on blood pressure changes and its associated factors in HAART naive HIV-infected patients in north eastern Nigeria. *Archives of Applied Science Research*. 2012;4(3):1447-52.
38. Kamga H, Assob J, Njunda A, Fon PN, Nsagha D, Atanga M, et al. The kidney function trends in human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS) patients at the Nylon District Hospital, Douala, Cameroon. *Journal of AIDS and HIV Research*. 2011;3(2):30-7.
39. Reid A, Stöhr W, Walker AS, Williams IG, Kityo C, Hughes P, et al. Severe renal dysfunction and risk factors associated with renal impairment in HIV-infected adults in Africa initiating antiretroviral therapy. *Clinical infectious diseases*. 2008;46(8):1271-81.
40. Wools-Kaloustian K, Gupta SK, Muloma E, Owino-Ong'or W, Sidle J, Aubrey RW, et al. Renal disease in an antiretroviral-naive HIV-infected outpatient population in Western Kenya. *Nephrology Dialysis Transplantation*. 2007;22(8):2208-12.
41. Struik G, Den Exter R, Munthali C, Chipeta D, Van Oosterhout J, Nouwen J, et al. The prevalence of renal impairment among adults with early HIV disease in Blantyre, Malawi. *International journal of STD & AIDS*. 2011;22(8):457-62.
42. Obirikorang C, Osakunor DNM, Ntaadu B, Adarkwa OK. Renal function in Ghanaian HIV-infected patients on highly active antiretroviral therapy: a case-control study. *PloS one*. 2014;9(6):e99469.

43. Agbaji OO, Onu A, Agaba PE, Muazu MA, Falang KD, Idoko JA. Predictors of impaired renal function among HIV infected patients commencing highly active antiretroviral therapy in Jos, Nigeria. *Nigerian Medical Journal*. 2011;52(3):182.
44. Msango L, Downs JA, Kalluvya SE, Kidenya BR, Kabangila R, Johnson Jr WD, et al. Renal Dysfunction among HIV-Infected Patients Starting Antiretroviral Therapy in Mwanza, Tanzania. *AIDS (London, England)*. 2011;25(11):1421.
45. Aggarwal HK, Jain D, Dahiya S, Jain P, Pawar S. Evaluation of renal profile in asymptomatic HIV patients with special reference to proteinuria.
46. González-Buitrago JM, Ferreira L, Lorenzo I. Urinary proteomics. *Clinica Chimica Acta*. 2007;375(1):49-56.
47. Navaratnarajah A, Jackson SH. The physiology of ageing. *Medicine*. 2017;45(1):6-10.
48. Tourret J, Deray G, Isnard-Bagnis C. Tenofovir effect on the kidneys of HIV-infected patients: a double-edged sword? *Journal of the American Society of Nephrology*. 2013;24(10):1519-27.
49. Ray AS, Fordyce MW, Hitchcock MJ. Tenofovir alafenamide: a novel prodrug of tenofovir for the treatment of human immunodeficiency virus. *Antiviral research*. 2016;125:63-70.
50. Cheesbrough M. District Laboratory Practice in Tropical Countries, 2nd edition update (part 1). 2012:314-5.
51. Organziation WH. Chronic diseases and health promotion: Stepwise approach to surveillance (STEPS). 2010.

12. ANNEX

Annex I. English version of patient's information sheet

Title of the research project: Prevalence of Chronic kidney disease and associated factors among HAART naïve and HAART experienced HIV infected individuals attending at the University of Gondar Hospital, Northwest Ethiopia.

Name of investigator: Shibihon Debebe (BSc, MSc candidate in Clinical chemistry)

Name of the organization: Department of clinical chemistry, School of Biomedical and Laboratory sciences, College of Medicine and health science, University of Gondar.

Introduction: You are invited to participate as study participant in a research which is going to conduct by the indicated MSc candidate from University of Gondar. Your participation is voluntarily. The research teams include one principal investigator, sample collectors, 2 advisors from University of Gondar. Please take as much time as you needed to read or listen the information sheet.

Purpose of the research project: You are requested to take part in this study because we are trying to learn more about the prevalence of Chronic kidney disease and associated factors among HAART naïve and HAART experienced HIV infected individuals attending at University of Gondar Hospital and for better disease management and prevention planning and also for providing information to healthcare provider as well as program designers.

Procedure: In order to perform the indicated study you are invited to take part in this project. If you are willing to participate, you need to understand the purpose of the study and give your consent then you will be interviewed for the issues and you will give laboratory sample related with this study. Laboratory sample include collection of 5 ml of venous blood by laboratory technologists for the determination of creatinine, glucose and urea. In addition 15 ml of urine sample will be collected for determination of urine albumin. The required clinical data, socio-demographic data and anthropometric measurements will be collected by experienced nurse by using semi-structured questionnaire. It will take about 10-15 minutes to fill the questionnaire.

Potential risks and discomforts: there are no anticipated risks in participating but you may experience small pain during blood collections.

Potential benefits to subjects and/or community: - Measuring serum creatinine only may not provide enough information about the status of the kidney except at end stage renal disease, but estimating GFR among suspected patient for CKD such as HIV infected individuals important for early diagnosis and for better treatment since it consider age, sex, body surface area and race. Thus, the result of the study will be beneficial to know your kidney status and associated risk factors and also to get an early treatment before reaching end stage renal disease, this also indirectly benefit the community.

Compensation for participation: - You will not receive any payment for your participation in this study.

Confidentiality: - There is no sensitive issues that you will be asked related with your social disability but any information that is obtained in connection with this study and that can be identified will remain confidential

Participation and withdrawal: - You can choose whether to be part of the study or not. You may withdraw at any time without consequences of any kind. You may also refuse to give any sample and/or information.

Person to contact:

If you have any question you can contact use any of the following (investigator and advisors) and you may ask at any time you want.

Shibihon Debebe (BSc, MSc candidate) telephone: 0912356542.

KetselaYirdaw (MSc), Lecturer at University of Gondar.

Daniel Asemelash (MSc), Lecturer at University of Gondar.

<u>የምርምሩ የአማርኛ ትርጉም አጭር መግለጫ</u>

<u>የምርምሩርዕስ</u>-ረዘም ላለ ጊዜ የቆየን የኩላሊት በሽታን መጠንና በሽታውን ሊያመጡ የሚችሉ ምክኒያቶችን ከኤችአይቪ በሽተኞች ላይ ማጥናት፡፡

<u>የተመራማሪዉ ስም</u>- ሽቢሆን ደበበ (ባችለር ሳይንስ ድግሪ፣የማስተር ደግሪ እጩ)

<u>የተቋሙ ስም</u>- ጎንደርዩ ኒቨርሲቲ ሕ.ጤ.ሳ.ኮ የባዮሜዲካል እና ላቦራቶሪ ሳይነስ ትምህርት ክፍል፡፡

<u>መግቢያ</u>፡- እርስዎ በተጠቀሰው የጎንደር ዩኒቨርስቲ የማስትርስ ተማሪ ጥናት ላይ እንዲሳተፉ ተጋብዘዋል፡፡ የእርስዎ ተሳትፎ በፍቃደኝነት ላይ የተመሰረተ ነው፡፡ ጥናቱም አንድ ዋና ተመራማሪ፣ ሁለት የተመራማሪው እረዳቶችን እና መረጃ ሰብሳቢዎችን ያካተተ ነው፡፡ እባክዎን በቂ ሳዓት ወስደው የምርምሩን መግለጫ ያንብቡት፡፡

<u>የጥናቱ አላማ</u>፡-እርስዎ በዚህ ጥናት እንዲሳተፉ የተደረገበት ምክንያት ረዘምላለ ጊዜ የቆየን የኩላሊት በሽታን መጠን እና እንዲሁም በሽታውን ሊያመጡ የሚችሉ ምክንያቶችን በጎንደር ዩኒቨርስቲ ኤች አይ ቪ በሽተኞችን ለማጥናት ነው፡፡ ጥናቱ የሚሠጠውን መረጃ መሰረት በማድረግ ይህ በሽታ የሚያደርሰውን ተጽኖ ለመከላከልና ህክምና አሰጣጡን ለማሻሻል ነው፡፡

<u>ዝርዝር ተግባራት</u>፡- መጀመሪያ እርስዎ በጥናቱ ለመሣተፍ ይጠየቃሉ፡፡ በጥናቱ ለመሳተፍ ፍቃደኛ ከሆኑ በሓኋላ እርስዎ ጥናቱን በተመለከተ አንዳነድ ቃለ-መጠይቆችን እና የላቦራቶሪ ናሙናዎችን እንዲሰጡን ይጠየቃሉ፡፡

ቃለ መጠየቁም ስለ ጥናቱ በሰለጠነ የጤና ባለሙያ (ነርስ) እና የላቦራቶሪ ናሙዎችን ልምድ ባለዉ የላቦራቶሪ ባለሙያ ይሰበሰባል፡፡ የላቦራቶሪ ናሙናምዉም 5 ሚሊሊትር ደም እና 15 ሚሊሊትር ሽንት ያካትታል፡፡

<u>የሚመጡ መጠነኛ ጉዳቶች</u>፡- እርስዎ በጥናቱ በመሳተፍዎ የሚደርስብዎት አደገኛ ጉዳት የለም፡፡ ይሁን እንጅ ደም በሚወሰድበት ጊዜ መጠነኛ የሆነ የመርፌ ህመም ስሜት ሊኖር ይችላል፡፡

ጥናቱ የሚሰጠው ጠቀሜታ፡- በደም ውስጥ ያለውን የኬራቲን መጠን መለካት ብቻውን ስለኩላሊት ጤንነት ጥሩ የሆነ መርጃ ለመስጠት አያስችልም፡፡ ነገር ግን በሽታው ያጠቃቸዋል ተብሎ ከሚጠረጠሩ በሽተኞች መካከል ለምሳሌ ያክል ኤችአይቪ በሽተኞችን የኩላሊት የማጣራት አቅም መለካት በሽታውን ቶሎ ለማወቅ እና የተሸለ ህክምና ለማግኘት ይርዳል፡፡

ስለሆነምጥናቱሊሰጥዎትየሚችለውዋናጥቅምየኩላሊትዎትንጤንነትእናይህንበሽታሊያመጡየ ሚችሉትንተጓዳኝምክኒያቶችንለማወቅይርዳል፡፡ ይህ ማለት በሌላ አነጋገር በማህበረሰቡ ከፍተኛ ጥቅም ይሠጣል ማለት ነው፡፡

የጥናቱ ተሳታፊ ካሳ፡- አርስዎ በጥናቱ ዉስጥ በመሳተፍዎ ምንም አይነት ክፍያ አይሰጠዎትም፡፡

የተሳታፊን ሚስጥር ስለመጠበቅ፡- እርስዎ በጥናቱ ዉስጥ በመሳተፍዎ ምንም አይነት ግለ-ስብዕናን የሚነካ መረጃ አይጠየቁም፡፡ ነገር ግን ጥናቱን በተመለከተ የሚወሰዱ መረጃዎችን በሚስጥራዊነት ይጠየቃሉ፡፡

በጥናቱ ስለመቀጠልና ስለማቋረጥ፡- እርስዎ በጥናቱ ለመሳተፍ በፍቃደኝነት ተጠይቀዋል፡፡ ከዚህም ባሻገር እርስዎ በጥናቱ መሳተፍ ና አለመሳተፍ የሚያስችል መብት አለዎት፡፡

የተመራማሪዉ አድራሻ

ሽቢሆን ደበበ፤ የሞባይል ቁጥር፡0912356542፣ጎንደርዩኒቨርሲቲሆስፒታል

የተመራማሪዉ አማካሪዎች አድራሻ

ቀፀላ ይርዳዉ (ማስተርስ ሳይንስ ዲግሪ)፡ ጎንደርዩኒቨርቲ

ዳንኤል አስመላሽ (ማስተርስ ሳይንስ ዲግሪ)፡ ጎንደርዩኒቨርሲቲ

Annex II. English version of Study participants consent form

I the undersigned individual has been well informed about the objectives of the study entitled "Prevalence of Chronic kidney disease and associated risk factors among HAART naïve and HAART experienced HIV infected individuals attending at the University of Gondar Hospital, Northwest Ethiopia''. I also informed that all information obtained at any course of the study is to be kept confidential. Moreover, I have also been well informed of my right to keep hold of, decline to cooperate and drop out of the study if I want and none of my actions will have any bearing at all on my overall health care and hospital access. Therefore, with full understanding of the situations I agree to give the entire necessary information and blood and urine sample for laboratory analysis.

Name……

Signature……

<u>የስምምነት ቅፅ የአማርኛ ግልባጭ</u>

እኔ ከዚህ በታች የፈረምኩት ጥናቱ በግልፅ ቋንቋ ተብራርቶልኝና ተረድቸ ነዉ። የጥናቱን አላማ ተረድቸ ለተመራማሪዉ አስፈላጊዉን መጠይቅና የደም እና ሽንት ናሙና ለመስጠት ተስማምቻለሁ። በጥናቱ ለመሳተፍ የወሰንኩት በራሴ ያለማንም ተፅዕኖ ሲሆን በማንኛዉም ጊዜ ጥቅሜ ሳይጓደልብኝ ከጥናቱ መዉጣት እንደምችል ተረድቻለሁ እንዲሁም ጥናቱን በተመለከተ የሚወሰድ መረጃ በሚስጥር እንደሚያዝ ተነግሮኛል።

የጥናቱ ተሳታፊ ስም …… ፊርማ……ቀን……

Annex III.Questionnaire prepared to assess the prevalence of CKD and associated factors among HAART naïve and HAART experienced HIV infected individuals at University of Gondar Hospital ART clinic, Northwest, Ethiopia.

Instruction: Please write and encircle the number in front of the choices that exactly fits the status

Part I. <u>Socio-demographic characteristics</u>

wo	**Code----------**		
	Question	Response	Code
1	Age	_____ years old	
2	Sex	Male Female	1 2
3	Ethnicity	Amhara Tigre Oromo Others----------	1 2 3 4
4	Religion	Orthodox Christian Muslim Protestant Others (specify)_____	1 2 3 4
5	Residence	Urban Rural	1 2
6	Current Marital status	Single Married Widowed Divorced Separated	1 2 3 4

7	Educational status	No education Primary school Secondary school Higher education	1 2 3 4
8	Occupation	Housewife Day laborer Government worker Private worker Merchant Farmer Other(specify)_____	1 2 3 4 5 6 7
9	Level of income per month in birr	_____Ethiopian Birr	
10	Smoking habit	Non- smoker Current smoker Ex- smoker If you have history of smoking, number of cigarettes each day_____	1 2 3
11	Have you ever consumed any alcohol such as beer, wine, spirits ,"tela","areki" in the last 1 month?	No Yes If yes, how much do you drink per day……	1 2 3
12	Do you have sleeping disorder	No Yes	1 2

Part II. **<u>Questions related to clinical & anthropometric measurements</u>**

13	Height in cm	_____cm	
14	Weight in Kg	_____kg	
15	Systolic blood pressure	---------- mmHg	
16	Diastolic blood pressure	---------- mmHg	
17	Blood pressure in mmHg	_____mmHg	
18	Do you use blood pressure-lowering medication?	No Yes	1 2
19	Diabetes mellitus	Absent Present	1 2
20	Type of DM	Type one Type two	1 2
21	Duration of DM	----------	
22	Do you have Family history of Kidney disease?	No Yes	1 2
23	Did you get AKI in the past	No Yes	1 2
24	Did you get kidney stone in the past	No Yes	1 2

25	Duration of HIV after diagnosis	----------	
26	WHO HIV stage	Stage I Stage II Stage III Stage IV	1 2 3 4
27	Duration of ARV treatment	----------	
28	Antiretroviral regimen	First line regimen Second line regimen	

Interviewer name _____Signature_____date_____

Thank you very much for your participation!!

ቃለ-መጠይቅ

ይህ ቃለ መጠየቅ የተዘጋጀው ረዘም ላለ ጊዜ የቆየን የኩላሊት በሽታን መጠንና በሽታውን ሊያመጡ የሚችሉ ምክኒያቶችን በጎንደር ዩኒቨርስቲ ሆስፒታል የኤችአይቪ በሽታ በሚታከሙ እና ለመታከም በሚመጡ በሽተኞች ላይ ለማትናት ነው።

እባክዎት እርስዎን የሚወክለውን አማራጭ ያክብቡ (በጽሁፍ ይግለጽልን)።

I. **አጠቃላይ ማህበራዊ መረጃ መጠይቅ**

ተ.ቁ	**የሚስጥርቁጥር----------**		
	ጥያቄ	**መልስ**	Code
1	**እድሜ**	_____ **ዓመት**	
2	**ፆታ**	**ወንድ** **ሴት**	1 2
3	**ብሄርዎ**	**አማራ** **ትግሬ** **ኦሮሞ** **ሌሎች**	
4	**ሀይማኖትዎ**	**ኦርቶዶክስ** **ሙስሊም** **ፕሮቴስታንት** **ሌሎች**	1 2 3 4
5	**የመኖሪያ አካባቢዎ**	**ከተማ** **ገጠር**	1 2
6	**የጋብቻ ሁኔታዎ**	**ያላገባ** **ያገባ** **የሞተት/ባት** **የተፋታ/ች**	1 2 3 4

7	የትምህርት ደረጃዎ	ያልተማረ የመጀመሪያ ደረጃ ያጠናቀቀ ሁለተኛ ደረጃና ከዚያ በላይ ከፍተኛ የትምህርት ደረጃና ከዚያበላይ	1 2 3 4
8	የስራ ሁኔታዎ	የቤት እመቤት የቀን ሰራተኛ የመንግስት ሰራተኛ የግል ስራ ነጋዴ ገበሬ ሌሎች (ይግለፁ)……	1 2 3 4 5 6 7
9	የወር ገቢዎ በብር	……ብር	
10	ሲጋራ ያጨሳሉ?	አላጨስም አጨሳለሁ አቁርጫለሁ ሲጋራ አጭሰዉ የሚያውቁ ከሆነ በቀን ምን ያክል ያጨሳሉ?_____	1 2 3
11	አልኮል መጠጥ (ቢራ ÷አረቄ- ÷ ጠላ÷ ጠጂ÷ ሌሎችም) ይጠጣሉ?	አልጠጣም አዎ መልስዎ አዎ ከሆነ በቀን ምን ያክል ይጠጣሉ? _____	1 2
12	የእንቅልፍ መዛባት በሽታ ገጥመዎት ያዉቃሉ?	የለም አዎ	1 2

II. የክሊኒካልና አንትሮፖሜትሪክ ልኬታን የሚመለከት መጠይቅና ልኬታ

13	ቁመት በሴንቲሜትር	_____ሴንቲሜትር	
14	ክብደት በኪሎግራም	_____ኪሎግራም	
15	ሲይስቶሊክ የደም ግፊት	----------ሚሊሜትርሜርኩሪ	
16	ዳይስቶሊክ የደም ግፊት	----------ሚሊሜትርሜርኩሪ	
17	የደም ግፊት በሚሊሜትር ሜርኩሪ	_____ሚሊሜትርሜርኩሪ	
18	የግፊት መዳኒት ይወስዳሉ?	አልወስድም አዎ	1 2
19	ስኳር በሽታ አለበዎት	አይደለም አዎ	1 2
20	የስኳር በሽታው አይነት	1.አንደኛው አይነት 2.ሁለተኛው አይነት	1 2
21	የስኳር በሽታ ከያዘወት ምን ያክል ጊዜ ሆነዎት?	_____	
22	ከቤተሰብዎት መካከል የኩላሊት በሽታ ታሞ የሚያውቅ አለ?	የለም አዎ	1 2
23	ፈጣን የኩላሊት በሽታ ታመዉ ያውቃሉ?	የለም አዎ	1 2
24	የኩላሊት ጠጠር ታመዉ ያዉቃሉ?	የለም አዎ	1 2

25	ኤችአይቪ በደሞ ዉስጥ መኖሩን ካውቁ ምን ያህል ግዜ ሆኖዎት?	----------	
26	የአለም አቀፍ የጤና ድርጅት ኤችአይቪ ደረጃ	ደረጃ 1 ደረጃ 2 ደረጃ 3 ደረጃ 4	1 2 3 4
27	የኤችአይቪመድሃኒትመዉሰድከጀመሩምንያህልግዜሆኖዎት?	----------	
28	የሚወስዱት የመድህኒት አይነት ምን አይነት ነዉ?		

ቃለመጥቁን ያስሞላዉ ስም_____ፊርማ _____ቀን_____

ላደረጉልን ተሳትፎ እጅግ በጣም እናመሰግናለን !!!

Annex IV. Check list for exclusion criteria of study participants

The patients who fulfilled the following characteristics were excluded from the study

1. Was the patient pregnant? A. Yes B. No
2. Was the patient amputee? A. Yes B. No
3. Was the patient years < 18 and >65? A. Yes B. No

Annex V. Laboratory procedures for blood and urine specimen collection and processing

I. Procedures for blood specimen collection and processing

Blood samples was taken from subjects by vein puncture into sterile clinical chemistry tubes with serum separator gel.

1) A sterile, dry 5ml plastic syringe was selected and attached to an appropriate needle.
2) A tourniquet wastied on the upper arm of the patient and was asked to make a fist.
3) Cotton wool soaked in 70% alcohol wasused to clean (sterilize) the skin for vein puncture.
4) Vein puncture was made with the bevel of the needle appropriately angled. A steady withdrawal of the plunger of the syringe was very necessary to prevent injuring the vein.
5) About 5mls of blood was collected before tourniquet was removed.
6) The needle was removed carefully and the puncture site pressed with a piece of cotton wool to stop bleeding.
7) The needle from the syringe was carefully disposed off properly.
8) The site of vein puncture was inspected for bleeding. A piece of cotton wool was placed on the site and a plaster placed on it.
9) The blood was transferred to the test tube.
10) The sample was allowed to stand to clot and centrifuge at 3000-5000rpm for 5 minutes.
11) Serum sample was separated and placed in neck tube, stored at -20^0c until processing (50).

II. Procedures for Urine specimen collection and processing

1. 15 ml of freshly voided urine was collected by clean,dry container.
2. The container was labeled by patient code.
3. Within 30 minute of collection the reagent area of the strip was dipped briefly into the specimen.
4. Excess urine was removed by tapping or drawing the edge of the strip along the rim of the urine container.
5. The color wascompared that developed with the color chart supplied by the manufacturer and report as indicated on the chart.

Annex VI. Laboratory methods, principles and procedures to determine biochemical tests

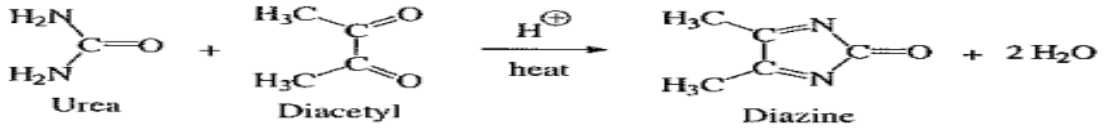
Chemical Jaffe reaction method to determine serum creatinine

In the Jaffe reaction, creatinine reacts with picric acid in an alkaline environment to generate an orange-red product and the intensity of color generated is directly proportional to creatinine concentration. The absorbance was read at wave length of 490-500nm according to manufacturer manual. The result was reported in mg/dl.

Creatinine +picric acid $\xrightarrow{\text{alkaline environment}}$ orange-red product

Chemical method to determine Urea

Urea in sample reacts directly with reagent called diacetylmonoxime which cause a color change that can be spectrophotometrically measured at 540nm and the increase intensity of the color is directly proportional to the amount of urea in the sample .The result was reported in mg/dl.



Colorimetric reagent strip (dipstick) test for urine protein detection

It is based on the ability of albumin to alter the color of some acid-base indicators without altering the P^H .when an indicator, such as tetra bromophenol blue is buffered at p^H 3, it is yellow in solution without albumin but it was blue in the presence of increasing amount of albumin. The result was reported qualitatively.

CD4 count

Principle: - When whole blood is added to the reagents, fluorochrome - labeled antibodies in the reagents bind specifically to lymphocyte surface antigens, and a fluorescent nuclear dye binds to the nucleated blood cells. After a fixative solution is added to the reagent tubes, the sample is run on the instrument. During sample acquisition, the cells come in contact with the laser light, which causes the fluorochrome-labeled cells and fluorescently dyed cells to fluoresce. This fluorescent light provides the information necessary for the instrument to identify and count the lymphocytes and CD4 lymphocytes. In addition, the reagent tubes also contain a known number of fluorescent reference beads. A precise volume of whole blood is stained directly in the reagent tube. The software automatically identifies lymphocyte populations and calculates the CD4 counts (cells/mm^3) by comparing cellular events to bead events and result printed immediately after sample was run.

Procedures:

1. The tab of each reagent tube was labeled with the patient accession number or number that identifies the tube of blood.
2. Each tube was vortexed upside down for 6 seconds and upright for 6 seconds.
3. Each reagent tube was opened with the coring station.
4. EDTA blood tube was mixed (vortexed) for 5 to 10 times to make sure that the whole blood was adequately mixed
5. 50µl of whole blood was pipetted into the labelled reagent tube with the corresponding patient accession number.
6. The tube was caped and vortexed upright for 6 seconds and incubate for 30 minutes in the working station.

7. Each sample tube was uncapped and pipetted by 50µl of fixative solution into each tube.
8. The sample tubes was run on the BD FACS Count instrument within 48 hours of adding fixative.

Anthropometric and Blood pressure measurement

Anthropometric measurement (weight, height) was measured according to WHO guidelines by trained nurses (51). Height was measured to the nearest 0.5cm using standio-meter and weight was recorded to the nearest 0.1kg with the patient being bare footed and wearing light clothes using a balance. From weight and height measurements BMI was calculated as weight divided by height squared (kg/ m^2). Underweight, Normal weight, overweight and obesity was defined as a BMI<18.5 kg/m^2, 18.5- 24.9 kg/m^2, 25-29.9 kg/m^2 and ≥30 kg/m^2, respectively.

Blood pressure was taken by qualified personnel using an analogue sphygmomanometer and stethoscope. Measurements were taken from the upper arm with the hand at the heart level after the patient had been sitting for more than 5 minutes. Systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg or current use of blood pressure-lowering medication was used to define hypertension.

Declaration

I, the undersigned, clinical chemistry MSc student declare that this thesis is my original work for fulfillment of the requirements for degree of Master of Science in Clinical Chemistry.

Name: Shibihon Debebe Signature ----------

Place of submission: Department of Clinical Chemistry, School of Biomedical and Laboratory sciences, College of Medicine and Health Sciences, University of Gondar.

Date of submission: ----------

This thesis was submitted for examination with our approval as university advisors

Advisors

Name	Signature
1. Ketsela Yirdaw (MSc)	_____
2. Daniel Asmelash (MSc)	_____

Examiners

1._____ Sign _____Date_____

2._____Sign_____Date_____

Annex XII

ASSURANCE OF INVESTIGATOR

The undersigned agrees to accept responsibility for the scientific, ethical and technical conduct of this thesis and for provision of required progress reports as pre terms and conditions of the research and publications office of the University of Gondar.

Name of the student: _____

Date: _____ Signature: _____

Approval of the advisor (s)

Advisors name	Signature	Date
1. _____	_____	_____
2._____	_____	_____

_____